Agronomic Improvement of Argomulyo Soybean Variety [*Glycine max* (l) merr] through Induced Mutation by Gamma Irradiation in M_1 and M_2 Generation

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Abstract

Gamma ray irradiation, especially applied at low doses, is one of methods to produce plant genetic diversity by means of micromutation. The objectives of this research was to determine the radiosensitivity of Argomulyo variety of soybeans (Glycine max (L) Merr) by gamma ray irradiation and to estimate genetic variablity of the crop after irradiation. To determine the radiosensitivy, the seeds of Argomulyo variety were treated with gamma ray irradiation at 200 Gy, 400 Gy, 600 Gy, 800 Gy, and 1000 Gy. Curve-fit analysis was employed to find the Lethal Doses 50 (LD_{50}) value . To evaluate the genetic variablity after irradiation with gamma ray, seeds of Argomulyo was irradiated under LD_{50} at doses of 0 Gy, 50 Gy, 100 Gy, 150 Gy, and 200 Gy. The results showed that LD_{50} value was 457.17 Gy. The study found that for Argomulyo variety the LD_{20} was 490.93 Gy and the LD_{50} was 457.17 Gy. Genetic variability was observed in various agronomic characters of M2 generation after low dose of gamma ray irradiation treatment of 50, 100, 150 and 200 Gy. The highest genetic variability was found at 200 Gy for plant height, number of productive nodes, and number of pods. The variability of agronomic characters of soybean after irradiation was due to genetic factors. The highest heritability estimates was observed in plant height and number of productive pods. The succeding generation of putative mutants will be selected for productivity and drought tolerance.

Key words: *induced mutation, gamma ray irradiation, Argomulyo soybean variety*

Introduction

Target for soybean production in 2009 is 850.230 tons or increasing by 9,50 percents in compare to 2008 total production of 776.490 ton (Indonesian Bereau of Statistics, 2009). This is a challenge since the productivity of soybean in Indonesia is still 1.3 ton/ha with decreasing harvest since 1992. Therefore, an increase in soybean production should be achieved through both improvement in productivity and increase in soybean harvest area.

As self-pollinated plants, soybean will form homozygous genotypes resulting in low intrapopulation genetic variability and significantly high interpopulation genetic variability. Genetic variability will increase in nature as a consequence of mutation or crossing between different populations, although with low rate leading to low genetic variability of soybean (Jusuf, 2004). The low genetic variability of soybean in Indonesia is also due to the fact that soybean is not an indigenous plant.

Improvement of soybean genetic variability could be done by introduction, hybridization or mutation. Argomulyo soybean variety is one of introduced variety from Thailand in addition of variety like Bromo, Krakatau and Tambora (Hidajat *et al*., 2000). Improvement soybean genetic variability is very important for both productivity and adaptation to environmental stress such as drought resistance. Mutation breeding is one of methods that could be applied to improve genetic variability of soybean.

Induced mutation can be applied in plants by means of treatment with certain mutagenic materials to reproductive organs like seeds, stem cuts, pollens, rhizomes, etc. On the other hands, spontaneous mutation occurs slowly, so that frequency and spectrum of plants mutation should be induced with certain mutagenic materials, such as gamma ray irradiation (BATAN, 2006). Gamma ray irradiation is one approach to induce genetic variability in crops. In this study gamma ray irradiation is applied at low doses (micro mutation) which is expected to induce mutation of quantitative characters with little influence on chromosome changes in compare to macromutation using gamma ray irradiation at high doses.

The objective of this study was to determine radiosensitivity of Argomulyo soybean variety to gamma ray irradiation with micro irradiation level on growth and development of soybean plants at first generation (M_1) and to estimate genetic variability induced by low gamma irradiation doses in second generation (M_2).

Materials and Methods

The study was conducted at the Bogor Agricultural University from February to June 2009. The study consisted of two experiments (1) determination of radiosensitivity of soybean variety of Argomulyo and (2) estimation of genetic variability induced by gamma ray irradiation (M2 generation). The irradiation treatment was conducted at the Center for Biological Resources and Biotechnology, Bogor Agricultural Unversity and the field evaluation was conducted at the university farm of Bogor Agricultural University.

The experiment on estimation of radiosensitivity of Argomulyo soybean variety used seeds with water content of less than 10 %. The seeds were exposed to 200 Gy, 400 Gy, 600 Gy, 800 Gy, and 1,000 Gy of gamma rays with a ^{137}Cs ∂ source using IBL 437C type H Irradiator (CIS Bio International, France).

There were six treatments including control (0 Gy) each of which was replicated 16 times. The seeds were then planted in seedling trays and were evaluated for germination and mortality at second week after planting. The variables examined were (1) germination, (2) seedling height, and (3)seedling mortality. Lethal Dose 50 (LD_{50}) value was determined using Curve-fit Analysis, a program to determine the best model equation to mortality percentage from a population (Finney, 2000).

After LD_{50} was determined, seeds of Argomulyo soybean variety were irradiated with gamma ray of 50 Gy, 100 Gy, 150 Gy and 200 Gy doses (micro doses). A total of 200 seeds (M1) of each dose were planted in a 40 x 20 cm distance and evaluated for the effect of gamma ray irradiation on plant morphology including flower characters, leaf characters, plant height, number of branches, number of pods, number of unfilled pods and number of seeds per plants. At generation M1 each plant of each doses was harvested 10 pods per plants (restricted bulk) to be planted as M_2 seeds.

The M_2 seeds were planted with 2,000 seeds per dose in a 40 x 20 cm distance and evaluated for variability in agronomic characters including plant height, number of branches, number of pods, number of unfilled pods and seed weight per plants. The genetic variation at generation M_2 was calculated as follows.

$$\sigma^2 = \frac{(\sum x^2) - [(\sum x)^2 / n]}{n-1}$$

$$\sigma^2 M_2 = \sigma^2 p;$$

$$\sigma^2 p = \sigma^2 g + \sigma^2 e;$$

$$\sigma^2 g = \sigma^2 p - \sigma^2 e = \sigma^2 M_2 - \sigma^2 M_0,$$

where

σ^2 = variance

n = the number of population members

$\sigma^2 p$ = phenotypic variance

$\sigma^2 g$ = genotypic variance

$\sigma^2 e$ = environment variance

$\sigma^2 M_2$ = the variance of M_2 population

$\sigma^2 M_0$ = the variance of M_0 population (Argomulyo population as control)

The broad sense heritability of the characters was estimated as follows.

$$h_{bs}^2 = \sigma^2 g / \sigma^2 p$$ (Singh and Chaudhari 1977)

The criteria of heritability value is

- $h^2 > 0,5$: high heritability value
- h^2 between 0,2 – 0,5 : moderate heritability value
- $h^2 < 0,2$: low heritability value

The coefficient for genotypic variation is determined based on coefficient of genetic variation (CVG) (Singh and Chaudhari, 1977) as follows.

$$CVG = \left(\frac{\sigma_g}{\bar{x}}\right) x 100\%$$

where :

σ_g = genetic standard deviation

$\bar{X}$ = the mean value of characters

The highest CVG absolute value are specified from 100 % CVG relative to value of 100 %.

Results and Discussion

All irradiated seeds germinated and two weeks after gamma ray irradiation, the mortality of seedling was observed to determine radiosensitivity level of Argomulyo soybean variety. Table 1 shows mortality and seedling height at two weeks after planting.

Table 1. Germination percentage (%) and seedling height at two weeks after planting

No.	Doses (Gy)	Germination percentage (%)	Seedling height (cm)
1	0 (control)	100	14.7
2	200	81.25	10.3
3	400	87.50	7.8
4	600	81.25	4.1
5	800	75	2.3
6	1,000	87.5	1.6

Table 1 shows that irradiation treatment causes mortality in Argomulyo seeds. The mortality of seedlings of irradiated seeds started at 3 days after germination (Figure 1). Increasing doses of irradiation caused higher mortality rate in soybean seedlings. In rice, irradiation caused failure of germination, where germination percentage decreased after irradiated with gamma ray, but its degradation was not proportional to increase in dose level (Cheema and Atta, 2007; Shah, *et al.,* 2008).

Figure 1. Germination of Argomulyo soybean variety induced by gamma ray irradiation at 0, 200, 400, 600, 800 and 1,000 Gy

The curve of radiosensitivity for Argomulyo variety is shown at Figure 2. The mortality depends on irradiation doses according to equation model $Y(x) = a + bx + cx^2$, where Y(x) was logarithm of entire germination, x was radiation dose and a, b, c was equation parameter (Table 2). Based on analysis using *CurceExpert 1.3.*, this study indicates that LD_{20} was 490.93 Gy and LD_{50} was 457.17 Gy.

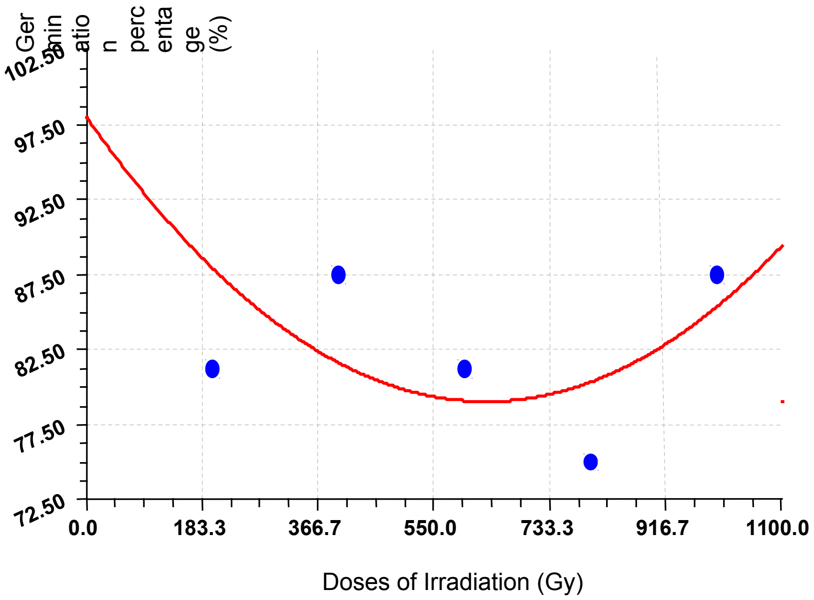

Figure 2. The effect gamma ray irradiation on germination percentage (%)

Figure 1 shows that plant growth was normal at 400 Gy, but the growth was inhibited and disturbed from 600 until 1,000 Gy. Visually, sensitivity level can be observed from response of plants, through plant morphology, sterility, and also LD_{50}. LD_{50} is the dose at which 50% mortality from irradiated population was found. From many studies close to irradiation, it has been known that generally desired mutation locates at range on LD_{50} or more precisely at a little under LD_{50} (Van Harten, 1998).

Table 2. LD50 of gamma ray irradiation for Argomulyo soybean variety

Variety	Model	Parameter Equation	R^2	LD_{50}
Argomulyo	$Y(x) = a + bx + cx^2$	a = 97.991 b = 0.0599 c = 0.0000473	0.81	457.13

Tah (2006) observed that mortality level of soybean plants increases linearly as doses increase. Sarkar *et al.* (1996) and Singh *et al.* (1997) observed that the influence of various doses of gamma ray irradiation on seed germination and seed strength was correlated linearly between mutagen doses and reduction of seed germination level.

There are some factors that influence radiosensitivity of plant to gamma ray irradiation. Plant morphology can influence resistance of cells to gamma ray irradiation. This condition also relates to water content of the seeds when irradiation is applied. Free radical produces collision in various directions, so that changes or mutations at DNA and cell level result, even lead to plant death (Datta, 2001).

Manjaya and Nandanwar (2007) reported that induced mutation and genetic variability was found in JS 80-21 soybean cultivar treated with gamma ray irradiation of 250 Gy, leading to released new variety of TAMS 98-21 having high seed production. Germination percentage is also influenced by condition such as plant vigor or seed size.

The gamma ray irradiation dose recommended by IAEA (Internasional Atomic Energy Agency) for soybean plants is 200 Gy. This dose is suitable to affect quantitative characters of the plants (Srisombun *et al.,* 2009).

M_0 soybean seeds treated with gamma ray irradiation may become mutated and heterozygous in some loci, thus the resulting M_1 seeds further planted to be M_1 plants usually exhibit reduced vigor and sterility. Observation of the M_1 plants of Argomulyo soybean variety after micro doses of gamma ray irradiation showed reduction in every characters, mainly plant height, as irradiation doses increase,. Table 3 shows that irradiation doses affected plant height. At 200 Gy, the growth of plants was shorter in compare to that of lower irradiation doses. In this study, Argomulyo soybean variety irradiated with 50, 100 and 150 Gy was higher than that of control. Nevertheless, along with the increase in irradiation doses, the means of plant height decreased.

Table 3. Means performance of characters at various irradiation doses

No.	Characters	Irradiation doses (Gy)				
		0	50	100	150	200
1	Plant height (cm)	34.20BC	38.00A	37.88A	36.42AB	30.47C
2	Number of branches	1.75A	2.35A	2.30A	1.90A	2.20A
3	Number of pods	27.65B	36.25A	35.75A	38.05A	29.20B
4	Number of unfilled pods	0.35A	0.35A	0.35A	0.35A	0.45A

This results were similar to those of Tah (2006) observing plant height at generation M_1 of mungbean plants [*Vigna radiata* (L.) Wilczek]. Plant height was reduced by gamma ray irradiation doses of 100 Gy, 200 Gy, 300 Gy and 400 Gy, where the highest reduction occured at 400 Gy. Shakoor *et al.* (1978) observed that treatment of 100 – 300 Gy did not significanty reduce plant height but that of 400 Gy caused plant dwarf.

Figure 3 shows that gamma ray irradiation influences changes in qualitative characters. Some plants show changes in leaf shape from oval (normal) to lanceolate. Some plants show changes from trifoliat to bifoliat and unifoliat above the first node. Changes in flower were observed in terms of colour from purple to white and failure in pod development. The changes in qualitative characters occur at some plants of irradiation doses of 150 and 200 Gy. Similar results were found in induced mutation with gamma ray irradiation on soybean (Manjaya and Nandawar, 2007) and mungbean (Sangsiri *et al.,* 2005), who observed the changes in shape and colour of leaf and flower where plant sterility was also generated.

Seeds harvested from M_1 plants are designated as M_2 seeds and planted as M_2 plants which are expected to show segregation in the mutated loci. Therefore, genetic variation should be observed in M_2 generation. In this study, observation was focused on agronomic characters such as plant height, yield components and yield.

The mean plant height of M_2 plants tends to increase with the increasing doses of irradiation (Table 4). However, at 200 Gy the mean plant height was lower than that at other doses. The mean number of productive branches was highest at 200 Gy and the lowest was found at 150 Gy. The highest mean number of productive nodes also occured at 200 Gy and the lowest occured at 50 Gy. The highest mean of productive pods occured at 200 Gy and the lowest mean occured at 150 Gy. The highest mean number of unfilled pods occured at 200 Gy and the lowest mean occured at 50 Gy. On the contrary to other agronomic characters, the highest mean of seeds weight occured at 50 Gy.

Genetic variability of agronomic characters of M_2 plants in various doses of irradiation can be seen at Table 4. The genetic variability which increased with the increase in irradiation doses were plant height, number of productive nodes and number of pods. For these characters, the highest genetic variability was observed at 200 Gy. In other characters, the genetic variability did not increase proportionally with doses as observed in number of productive branches and seed weight. The highest genetic variability of seed weight was oberved in 100 and 150 Gy.

(a). Trifoliate leaf and oval (normal)

(b). Bifoliat leaf and lanceolate

(c). Unifoliat leaf and lanceolate

(d). Flower rasim and bud (not expand)

(e). Purple flower (normal)

(f).White flower

Figure 3. Changes in morphological characters of Argomulyo soybean variety plants after gamma ray irradiation

The above observation on genetic variability shows that lower doses of irradiation may produce variability of desired characters. Sakin (2002) also observed mutation in quantitative characters of durum wheat at micro doses of gamma ray irradiation. The advantage of using low doses of irradiation is that it allows mutation of minor genes to be observed at later generation without deleterious mutation. Sangsiri *et al*. (2005) observed many deleterious mutation, like albino and changes in leaf shape, in mungbean after irradiation treatment of 500 Gy.

The variability of agronomic characters in soybean after irradiation with gamma ray are genetically controlled. The high genetic variation is very important in selection because genetic response to selection depends on the level of genetic variation (Hallauer, 1987). High broad sense heritability estimates were found in plant height and number of productive pods. This means that selection for improvement of both characters is promising to produce genotypes with desirable height and productivity. For other characters, the heritability estimates ranged from low to moderate. Similar results in mungbean were reported by Chaturvedi and Singh (1980) and Tah (2006). Sakin (2002) observed that the heritability values for some mutant populations in durum wheat depend on the characters under study.

Table 4. Genetic variation and heritability of M_2 plants at various doses of irradiation

No.	Characters	Irradiation Doses			
		50	100	150	200
1	Plant Height				
	$\bar{x}$	32.76	33.14	34.73	34.43
	σ^2p	12.953	13.492	24.824	28.060
	σ^2g	7.092	7.630	18.962	22.198
	h^2	0.547	0.565	0.764	0.791
	CVG (%)	8,129	8.334	12.536	13.683
2	Number of Productive Branches				
	$\bar{x}$	2.73	2.81	2.48	3.01
	σ^2p	2.572	1.055	1.237	0.951
	σ^2g	1.924	0.406	0.588	0.302
	h^2	0.748	0.385	0.475	0.317
	CVG (%)	50.785	22.645	30.810	18.276
3	Number of Productive Nodes				
	$\bar{x}$	17.24	18.00	17.28	19.02
	σ^2p	15.587	23.611	25.468	24.507
	σ^2g	1.105	8.611	12.468	14.507
	h^2	0.071	0.365	0.490	0.592
	CVG (%)	6.097	16.302	20.431	20.024
4	Number of Productive Pods				
	$\bar{x}$	39.08	38.13	38.07	43.19
	σ^2p	111.368	220.605	191.209	209.976
	σ^2g	36.013	145.251	115.855	134.622
	h^2	0.323	0.658	0.606	0.641
	CVG (%)	15.355	31.601	28.269	26.862
5	Number of Unfilled Pods				
	$\bar{x}$	1.40	2.53	2.60	3.05
	σ^2p	2.783	5.792	4.866	7.705
	σ^2g	0.131	3.140	2.214	5.053
	h^2	0.047	0.542	0.455	0.655
	CVG (%)	25.882	70.015	57.237	73.578
6	Seeds Weight				
	$\bar{x}$	10.93	9.85	9.47	10.17
	σ^2p	10.775	16.458	16.574	12.116
	σ^2g	0.033	5.716	5.832	1.374
	h^2	0.003	0.347	0.352	0.113
	CVG (%)	1.664	24.257	25.494	11.521

Conclusion

It was found that LD_{20} for Argomulyo variety was 490.93 Gy and the LD_{50} was 457.17 Gy. Genetic variability was observed in various agronomic characters of M_2 generation after low dose of gamma ray irradiation treatment with. The variability of agronomic characters of soybean after irradiation was due to genetic factors. The highest heritability estimates was observed in plant height and number of productive pods. The succeding generation of putative mutant will be selected for productivity and drought tolerance.

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